EXHIBIT A

Organic Chemistry

Third Edition

John McMurry
Cornell University

BEST AVAILABLE COPY

Brooks/Cole Publishing Company
Pacific Grove, California

Brooks/Cole Publishing Company
A Division of Wadsworth, Inc.

Printed in the United States of America

10 9 8 7 6 5 4 3 2

Library of Congress Cataloging in Publication Data

McMurry, John.
Organic chemistry / John McMurry. — 3rd ed.
p. cm.
Includes index.
ISBN 0-534-16218-5
1. Chemistry, Organic. I. Title.
QD251.2.M43 1992
547—dc20 91-40322
CIP

Sponsoring Editor: Harvey C. Pantzis
Editorial Assistant: Nancy Miaoulis
Production: Joan Marsh/Mary Douglas
Production Assistant: Scott Alkire
Manuscript Editor: Margaret Moore
Text Design: Nancy Benedict
Cover Design: Michael Rogondino
Cover Photo: MOZO Photo/Design
Chapter-Opener Illutrations: Pat Rogondino
Interior Illustration: John and Jean Foster, Dave Pauly
Typesetting: Jonathan Peck Typographers, Ltd.
Cover Printing: The Lehigh Press
Printing and Binding: R. R. Donnelley & Sons, Inc.
Photo, p. 1124: A. Lesk/SPL, Photo Researchers

PROBLEM

29.21 Show the labeled cleavage products you would expect to obtain if the following DNA segment were subjected to each of the four cleavage reactions:

^{32}P-A-A-C-A-T-G-G-C-G-C-T-T-A-T-G-A-C-G-A

PROBLEM

29.22 Sketch what you would expect the gel electrophoresis pattern to look like if the DNA segment in Problem 29.21 were sequenced.

PROBLEM

29.23 Finish assigning the sequence to the gel electrophoresis pattern shown in Figure 29.17.

29.17 Laboratory Synthesis of DNA

The development of genetic engineering techniques in the last two decades brought with it an increased demand for efficient chemical methods for the synthesis of short DNA segments. Ideally, whole genes might be synthesized in the laboratory and inserted into the DNA of microorganisms, thereby directing the microorganisms to produce the specific protein coded for by that gene—perhaps insulin or some other valuable material.

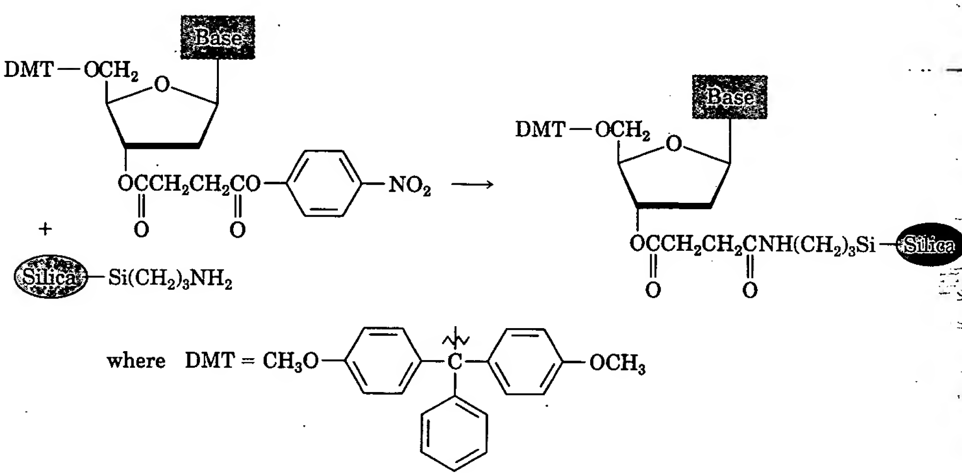
The problems of DNA synthesis are similar to those of protein synthesis (Section 27.11) but are considerably more difficult because of the structural complexity of the deoxyribonucleotide monomers. Each nucleotide has several reactive sites that must be selectively protected and deprotected at the proper times, and coupling of the four nucleotides must be carried out in the proper sequence. Despite these difficulties, some extremely impressive early achievements were recorded, most notably the synthesis by Khorana[4] in 1979 of the tyrosine suppressor tRNA gene from the bacterium *Escherichia coli*. Some 207 base pairs were assembled in an effort that required 10 years of work.

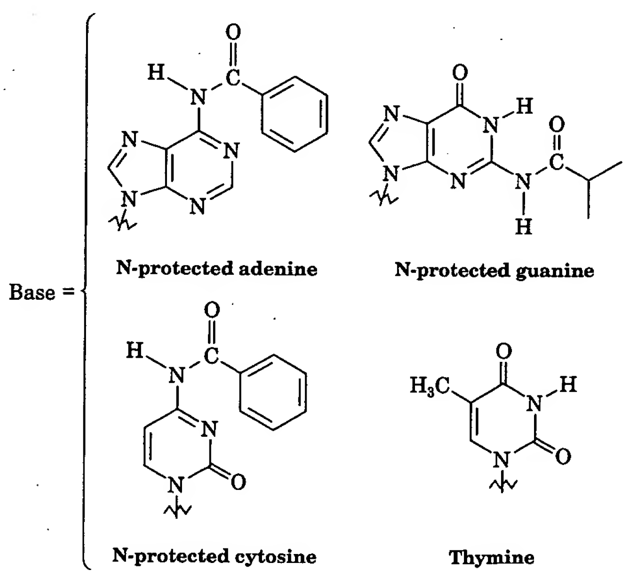
More recently, automated "gene machines" have become available, which allow the fast and reliable synthesis of DNA sequences 100–200 nucleotides long. These DNA synthesizers operate on a principle similar to that of the Merrifield solid-phase peptide synthesizer (Section 27.12). In essence, a protected nucleotide is covalently bound to a solid support, and one nucleotide at a time is added to the chain. When the last nucleotide has been added, the protecting groups are removed, and the synthetic DNA is cleaved from the solid support.

[4]Har Gobind Khorana (1922–); b. Raipur, India; Ph.D. University of Liverpool; professor, Massachusetts Institute of Technology; Nobel Prize in medicine (1968).

Step 1 in DNA synthesis involves attachment of a protected deoxynucleoside to a silica (SiO_2) support by an ester linkage to the deoxynucleoside's 3′ position. Both the 5′ hydroxyl and free amino groups on the heterocyclic bases must be protected to accomplish this attachment. Adenine and cytosine bases are protected by benzoyl groups, guanine is protected by an isobutyryl group, and thymine requires no protection. The deoxyribose 5′ hydroxyl is protected as its DMT ether, where DMT = *p*-dimethoxytrityl. Other protecting groups can also be used, but those mentioned here are common choices.

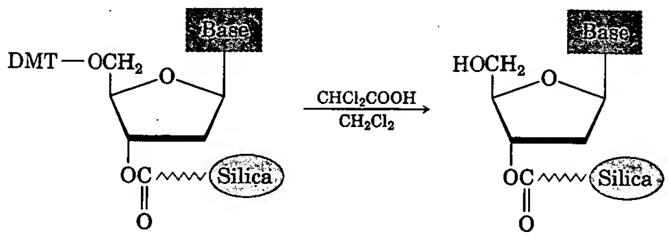
Step 1





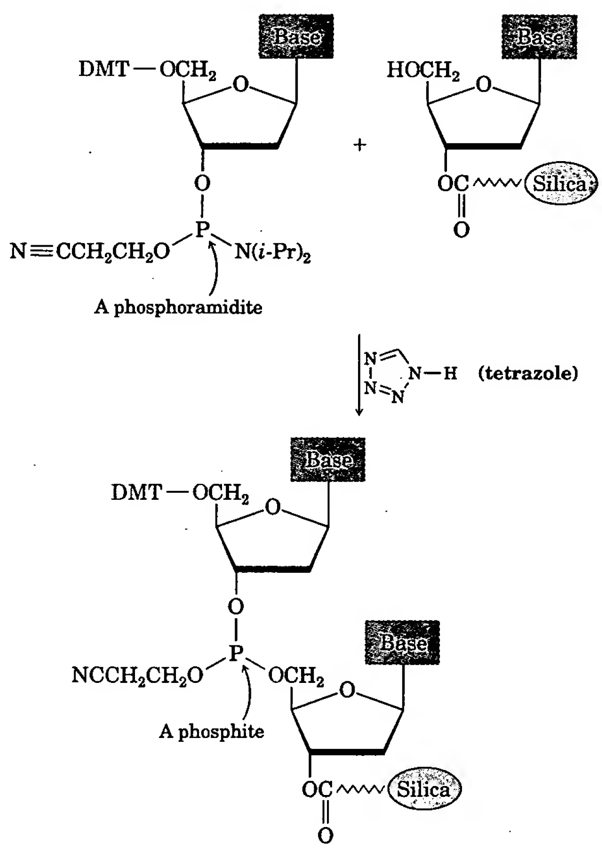
Step 2 involves deprotection of the 5′-deoxyribose hydroxyl by treatment with dichloroacetic acid in CH_2Cl_2 to remove the DMT group. The reaction proceeds rapidly by an S_N1 mechanism because of the stability of the tertiary, triply benzylic dimethoxytrityl cation.

Step 2



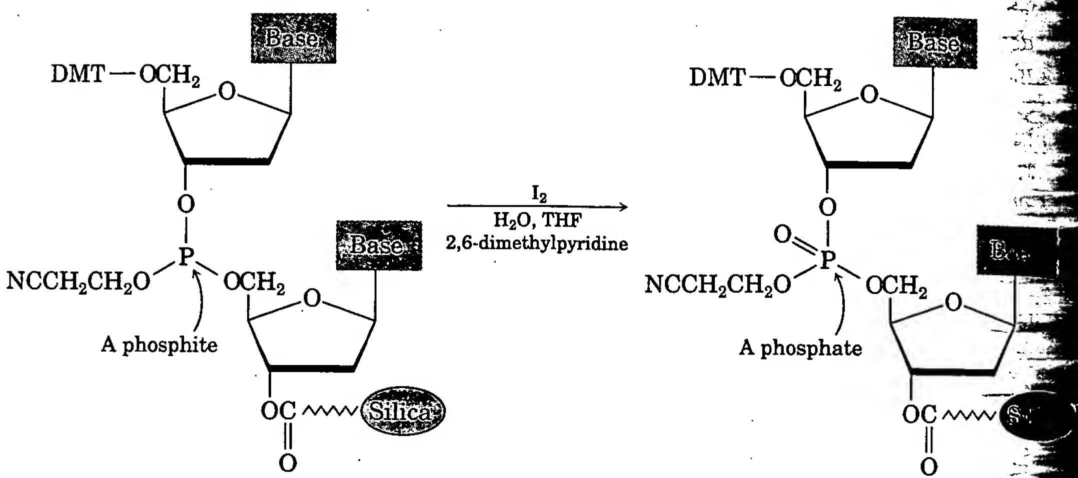
Step 3 involves coupling of the polymer-bonded deoxynucleoside with a protected deoxynucleoside containing a *phosphoramidite* group at its 3′ position. [A phosphoramidite has a trivalent phosphorus with the structure $R_2NP(OR)_2$.] The coupling reaction takes place in the polar aprotic solvent acetonitrile, requires catalysis by the heterocyclic amine tetrazole, and yields a *phosphite*, or trialkoxyphosphorus compound, $P(OR)_3$, as product. Note that one of the phosphorus oxygen atoms is protected by a β-cyanoethyl group. The coupling step takes place in better than 99% yield.

Step 3



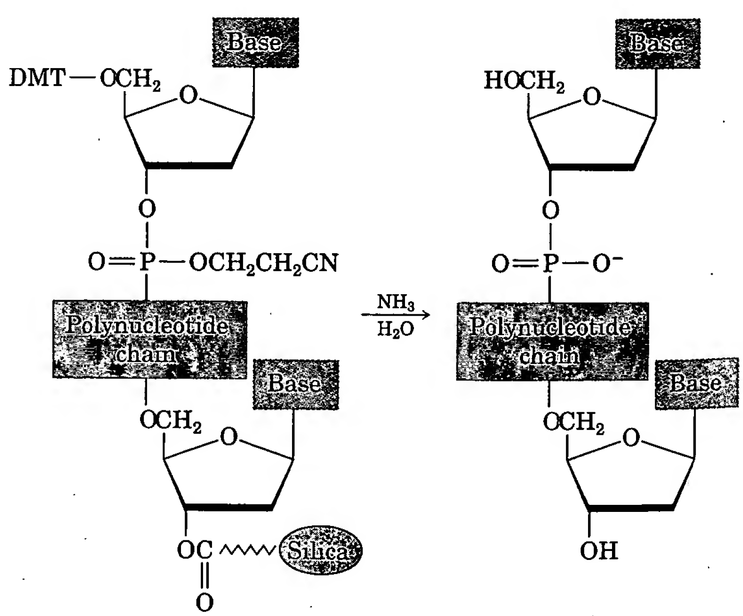
With the coupling completed, the phosphite product is then oxidized to a phosphate triester by treatment with iodine. The reaction is carried out in aqueous tetrahydrofuran in the presence of 2,6-dimethylpyridine.

Step 4



The cycle of steps—deprotection, coupling, and oxidation—is then repeated until a polydeoxyribonucleotide chain of the desired length and sequence has been built. The final step is to cleave all protecting groups from the heterocyclic bases and from the phosphates and to cleave the ester bond holding the DNA to the silica. All of these reactions are done at the same time by treatment with aqueous ammonia. Purification by electrophoresis then yields the synthetic DNA.

Step 5



PROBLEM

29.24 *p*-Dimethoxytrityl (DMT) ethers are easily cleaved by mild acid treatment. Show the mechanism, and suggest a reason why this ether cleavage is unusually easy.